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Stability Indicating Method Development For Simultaneous Estimation Of Carvedilol (Cdl) And Ivabradine (Ivd) By Hplc

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Abstract
A simple, accurate, and precise RP-HPLC method was developed and validated for the simultaneous estimation of Carvedilol (CDL) and Ivabradine (IVD) in bulk and pharmaceutical dosage forms. The chromatographic separation was achieved using a C18 column with a mobile phase consisting of a suitable buffer and acetonitrile in an optimized ratio. The method exhibited excellent linearity in the concentration range of 1–5 µg/ml for both CDL and IVD with correlation coefficients (r^2) of 0.9996 for each drug. System suitability parameters, such as retention time, tailing factor, and number of theoretical plates, were within acceptable limits, ensuring efficient system performance. The recovery study confirmed the accuracy of the method, with percent recoveries ranging between 97.13% and 98.72% for both drugs. The method also demonstrated excellent repeatability, day-to-day precision, analyst-to-analyst reproducibility, and robustness, with %RSD values consistently below 1.5%. Furthermore, forced degradation studies confirmed the stability-indicating nature of the method. This validated method can be effectively applied for routine quality control and stability testing of CDL and IVD in pharmaceutical formulations.
Keywords: *Carvedilol, Ivabradine, RP-HPLC, Method Validation, Recovery Study, Linearity, Repeatability, Stability-Indicating Method, Forced Degradation, Pharmaceutical Analysis*

INTRODUCTION

High-performance liquid chromatography (HPLC) has become an indispensable tool in the pharmaceutical industry for the qualitative and quantitative analysis of drugs, especially for stability studies. A stability-indicating method is an analytical procedure that accurately and precisely measures active pharmaceutical ingredients (APIs) without interference from degradation products, process impurities, excipients, or other potential impurities. These methods are crucial for establishing the stability profile of drugs and ensuring their efficacy and safety throughout their shelf life, as per ICH Q1A (R2) guidelines on stability testing of new drug substances and products [1]. Carvedilol (CDL) is a non-selective beta-adrenergic blocker with alpha-1 blocking activity, commonly prescribed for the treatment of hypertension, congestive heart failure, and left ventricular dysfunction following myocardial infarction [2]. It is classified as a BCS Class II drug due to its poor aqueous solubility and high permeability. Carvedilol undergoes extensive first-pass metabolism primarily by cytochrome P450 enzymes, and its degradation is influenced by acidic and oxidative stress conditions [3]. Ivabradine (IVD) is a selective sinus node inhibitor that acts by reducing the heart rate through inhibition of the If current in the sinoatrial node. It is used in the management of chronic heart failure and stable angina [4]. Ivabradine hydrochloride is known to be photosensitive and may degrade under alkaline and oxidative conditions [5]. The fixed-dose combination (FDC) of Carvedilol and Ivabradine has gained attention for its synergistic action in patients with heart failure and chronic stable angina. Hence, developing a robust and reliable HPLC-based simultaneous estimation method is vital for the quality control and stability assessment of such formulations. Several analytical methods have been reported individually for the estimation of Carvedilol and Ivabradine using UV spectroscopy [6,7], extractive spectrophotometry [8], and HPLC [9,10]. However, only a few methods have been documented for their simultaneous estimation, and even fewer studies provide a comprehensive stability-indicating HPLC method that can distinctly identify and separate the degradation products of both drugs. A well-validated stability-indicating HPLC method should be able to demonstrate specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ), as outlined in ICH Q2(R1) guidelines on analytical method validation [11]. It should also confirm the separation of active

components from potential degradants under stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic environments. The present study aims to develop and validate a simple, accurate, precise, and stability-indicating reverse-phase HPLC method for the simultaneous estimation of Carvedilol and Ivabradine in their combined tablet dosage form. The method is further subjected to **forced** degradation studies to ensure its suitability for detecting the drugs and their degradation products, thereby supporting stability testing and quality control processes in pharmaceutical environments.

MATERIAL AND METHODS

Selection of mobile phase

Initially to estimate CDL and IVD in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 10mM KH_2PO_4: Acetonitrile (pH 4.0 with OPA) in the ratio of 20:80v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials Acetonitrile was used as diluents.

Selection of separation variable

Table 1: Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5µ
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
10mM KH_2PO_4	20
Acetonitrile	80
Diluent	Acetonitrile
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 µl
Detection wavelength	254mm
Retention time	
Carvedilol	5.252 ± 0.3min
Ivabradine	7.654 ± 0.3min

Preparation of standard Stock solution Accurately weighed 10 mg of CDL and IVD was transferred into 10 ml volumetric flasks separately and dissolved in 5 ml of methanol and sonicate for 10 min., then volume was made up to 10 ml with Acetonitrile. Concentration of CDL and IVD in methanol was 1000µg/ml. (stock- A).

Preparation of Sub Stock Solution 1 ml of solution was taken from stock-A of CDL and IVD and transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent (Acetonitrile) to give concentration of 100µg/ml (Stock-B).

Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with (Acetonitrile). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml for drug. In same manner 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml IVD also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5µg/ml for CDL and 1-5µg/ml for IVD were prepared. All the solution were filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 254nm and it was repeat for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System suitability parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of CDL 5µg/ml and IVD 5µg/ml were injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of HPLC method development [11]

Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of CDL and IVD to preanalysed tablets powder. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to day was performed by analyzing 5 different concentration of the drug for three days in a week.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of tablets formulation

Tablet powder were weighed and ground to a fine powder; amount equal to 5mg IVD and 3.125 mg CDL was taken in 10 ml volumetric flask. Then 5ml of Acetonitrile was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with acetonitrile. After sonication filtration was done through 0.45µ membrane filter. Filtrate was collected and further diluted with methanol to get the final concentrations of drugs in the working range. The mean area of final dilutions was observed the concentrations were obtained from calibration curve method. The procedure was repeated for five times.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation:

10 mg of both the drugs were taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80°C. Samples were withdrawn and diluted to get 10µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Alkaline hydrolysis:

10 mg of both the drugs were taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80°C. Samples

were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Oxidative degradation:

10 mg of both the were taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Thermal degradation:

10 mg of both the drugs were taken into a petri dish and kept in oven at 50°C for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

RESULTS AND DISCUSSION

The developed analytical method for Carvedilol (CDL) and Ivabradine (IVD) was validated through various parameters including linearity, system suitability, accuracy, precision, and robustness. The results confirm that the method is reliable and suitable for routine quality control of both drugs.

The method exhibited excellent linearity over a concentration range of 1–5 µg/ml for both CDL and IVD with correlation coefficients (r^2) of 0.9996 each, indicating a strong linear relationship between concentration and detector response (Table 2).

The system suitability parameters such as retention time (RT), area under curve (AUC), number of theoretical plates, and tailing factor were within acceptable limits. CDL showed a mean RT of 5.251 min, while IVD had 7.654 min, confirming clear separation and system efficiency (Table 3).

Accuracy of the method was validated through recovery studies at three levels: 80%, 100%, and 120%. Recovery values for CDL ranged from 97.63% to 98.72%, and for IVD from 97.13% to 98.51%, with all % R.S.D. values below 1.1%, confirming good accuracy (Table 5).

The method demonstrated excellent repeatability across five concentrations for both drugs. The % R.S.D. values were consistently 0.012%, with very low standard deviation, indicating excellent precision under the same conditions (Table 6).

To test intermediate precision, day-to-day variation was studied over three days. For CDL, the % mean ranged between 93.00%–97.83%, and for IVD, between 95.67%–99.20%, showing the method is consistent over multiple days. Again, % R.S.D. values remained at 0.012%, showing high reproducibility (Table 7).

The method produced consistent results across different analysts and laboratories. For both drugs, % R.S.D. remained 0.012%, confirming the method is analyst-independent and reproducible (Table 8).

The Limit of Detection (LOD) and Limit of Quantification (LOQ) values were determined to assess the method's sensitivity. CDL showed LOD and LOQ of 0.15 µg/ml and 0.40 µg/ml, respectively, while IVD showed 0.20 µg/ml and 0.65 µg/ml, indicating high sensitivity (Table 9).

The method was successfully applied to marketed formulations. The amount of CDL found was 6.15 mg (98.40%) against the label claim of 6.25 mg, and IVD was 4.95 mg (99.00%) against the label claim of 5 mg. The low standard deviations confirm consistent performance (Table 10).

The stability of both drugs under various stress conditions was evaluated. CDL showed highest degradation under acidic conditions (13.2%), while IVD showed higher degradation under photolytic (9.8%) and alkaline (11.1%) conditions. However, both drugs retained more than 86% integrity under all conditions, proving method stability and selectivity (Table 11).

Table 2: Results of Linearity of Carvedilol (CDL) and Ivabradine (IVD)

Drug	Concentration (µg/ml)	Correl Coeff (r^2)	Slope (m)
CDL	1 - 5	0.9996	567.64
IVD	1 - 5	0.9996	607.65

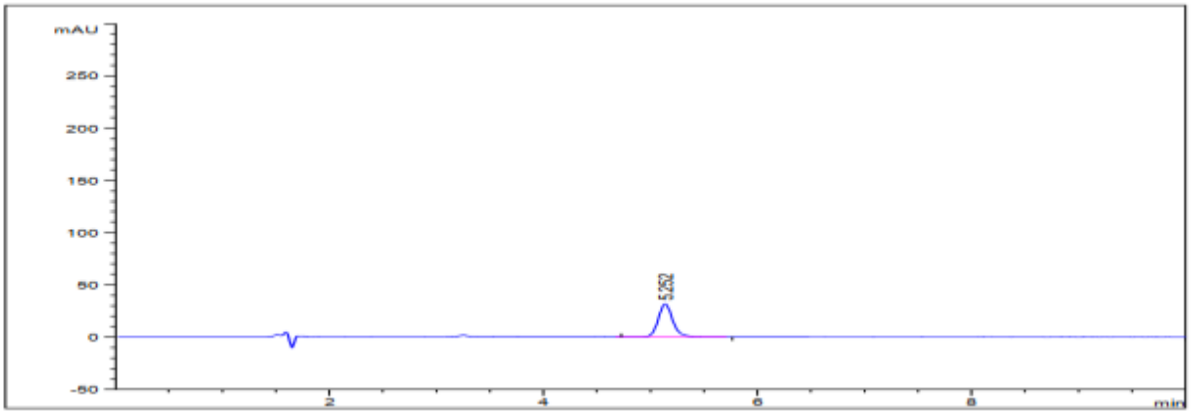

Figure 1: Chromatogram of CDL

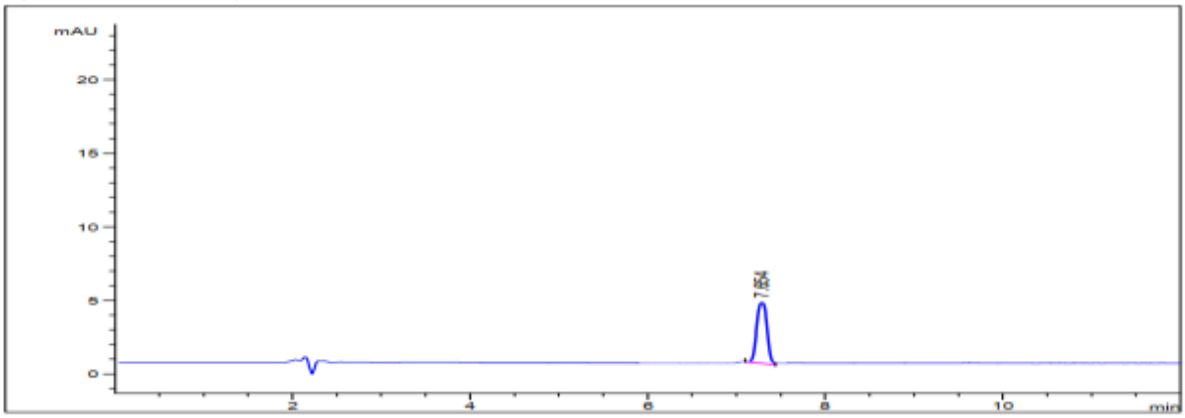

Figure 2: Chromatogram of IVD

Table 3: System Suitability Parameters of Carvedilol (CDL) and Ivabradine (IVD)

Carvedilol (CDL)				
System Suitability Parameter	RT	AUC	No. of Theoretical Plates	Tailing Factor
Mean	5.251	2856.686	3260.833	1.152
S.D.	0.004	11.150	26.393	0.017
% R.S.D.	0.076	0.390	0.809	1.496
Ivabradine (IVD)				
Mean	7.654	3028.080	2862.833	1.137
S.D.	0.005	8.456	51.316	0.015
% R.S.D.	0.068	0.279	1.793	1.325

Table 4: Response Ratio of Carvedilol (CDL) and Ivabradine (IVD)

S. No.	CDL			IVD		
	Conc. (µg/ml)	Area	Response Ratio	Conc. (µg/ml)	Area	Response Ratio
1	1	565.908	565.908	1	631.9	631.900
2	2	1161.31	580.656	2	1238.67	619.335
3	3	1674.54	558.180	3	1879.23	626.410
4	4	2256.19	564.048	4	2460.81	615.203
5	5	2856.69	571.337	5	3028.08	605.616

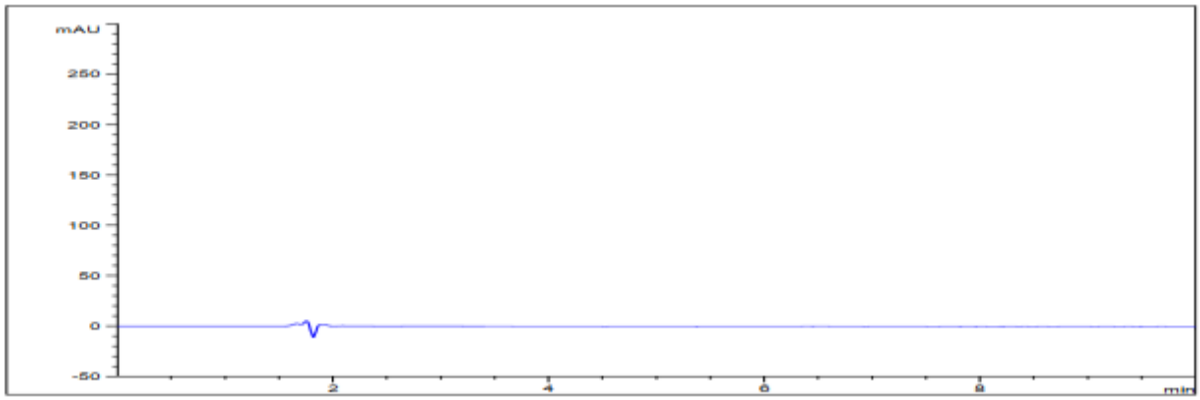

Figure 3: Chromatogram of Blank

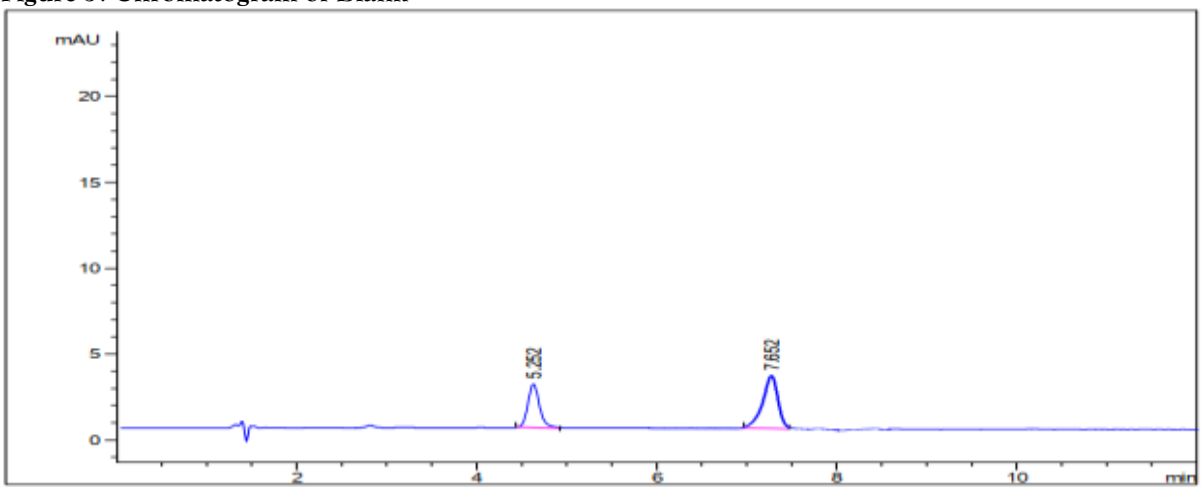

Figure 4: Chromatogram of CDL and IVD

Table 5: Results of Recovery study of Carvedilol (CDL) and Ivabradine (IVD)

Recovery Level	CDL			IVD		
	Mean	S.D.	% R.S.D.	Mean	S.D.	% R.S.D.
80% Level	98.72	0.663	0.672	98.51	0.625	0.634
100% Level	97.63	0.918	0.941	97.13	1.501	1.545
120% Level	98.42	0.924	0.939	98.51	1.072	1.089

* Mean of 3 replicate and 5 concentrations

Table 6: Results of Repeatability study of Carvedilol (CDL) and Ivabradine (IVD)

Concentration (µg/ml)	CDL				IVD			
	Mean	% Mean	S.D.	% R.S.D.	Mean	% Mean	S.D.	% R.S.D.
1	0.966	96.600	0.025	0.012	0.976	97.600	0.011	0.012
2	1.962	98.100	0.013	0.012	1.948	97.400	0.056	0.012
3	2.894	96.467	0.077	0.012	2.904	96.800	0.048	0.012
4	3.900	97.500	0.082	0.012	3.928	98.200	0.040	0.012
5	4.916	98.320	0.060	0.012	4.920	98.400	0.064	0.012

Table 7: Day-to-Day Variation of Carvedilol (CDL) and Ivabradine (IVD)

Concentration (µg/ml)	CDL							IVD						
	Day 1	Day 2	Day 3	Mean	% Mean	S.D.	% R.S.D.	Day 1	Day 2	Day 3	Mean	% Mean	S.D.	% R.S.D.
1	0.99	0.95	0.96	0.967	96.667	0.021	0.012	0.95	0.93	0.99	0.957	95.667	0.031	0.012

2	1.98	1.96	1.92	1.953	97.667	0.031	0.012	1.85	1.93	1.97	1.917	95.833	0.061	0.012
3	2.75	2.69	2.93	2.790	93.000	0.125	0.012	2.85	2.96	2.87	2.893	96.444	0.059	0.012
4	3.85	3.92	3.97	3.913	97.833	0.060	0.012	3.85	3.96	3.87	3.893	97.333	0.059	0.012
5	4.75	4.65	4.85	4.750	95.000	0.100	0.012	4.95	4.95	4.98	4.960	99.200	0.017	0.012

Table 8: Results of Analyst-to-Analyst Variation and Reproducibility of Carvedilol (CDL) and Ivabradine (IVD)

Parameters	Analyst-to-Analyst Variation		Reproducibility	
	CDL	IVD	CDL	IVD
% Mean	97.178	96.578	96.452	95.069
S.D.	0.054	0.072	0.056	0.082
% R.S.D.	0.012	0.012	0.012	0.012

Table 9: LOD and LOQ of of Carvedilol (CDL) and Ivabradine (IVD)

Name	LOD (µg/ml)	LOQ (µg/ml)
CDL	0.15	0.40
IVD	0.20	0.65

Table 10: Mean analysis of tablets formulation of of Carvedilol (CDL) and Ivabradine (IVD)

Name of Drugs	Label Claim (mg)	Amount Found (mg)	Mean	SD	%RSD
CDL	6.25	6.15	98.40	0.962	0.979
IVD	5	4.95	99.00	1.131	1.179

Table 11: Results of Forced Degradation Studies of of Carvedilol (CDL) and Ivabradine (IVD)

Stress Conditions	CDL		IVD	
	Drug Recovered (%)	Drug Decomposed (%)	Drug Recovered (%)	Drug Decomposed (%)
Standard drug	99.85	0	99.75	0
Acidic hydrolysis	86.65	13.2	92.25	7.5
Alkaline hydrolysis	92.32	7.53	88.65	11.1
Oxidative degradation	88.98	10.87	90.14	9.61
Photolytic degradation	97.78	2.07	89.95	9.8

CONCLUSION

The developed RP-HPLC method for the simultaneous estimation of Carvedilol (CDL) and Ivabradine (IVD) was found to be simple, precise, accurate, robust**,** and stability-indicating**.** The method showed excellent linearity over the concentration range of 1–5 µg/ml for both drugs, with correlation coefficients (r^2) of 0.9996, indicating a strong relationship between concentration and peak area. The method passed all system suitability parameters (Table 3), with satisfactory results in terms of retention time, theoretical plates, and tailing factor. Recovery studies at 80%, 100%, and 120% levels (Table 5) confirmed the method's accuracy, with percent recoveries within the acceptable range for both CDL and IVD. Precision was confirmed through repeatability, day-to-day, and analyst-to-analyst variations (Tables 6–8), showing low %RSD values, all below 1.5%, indicating excellent reproducibility and reliability. Moreover, the method was proven to be stability-indicating through forced degradation studies (Table 11), as it

successfully separated the degradation products from the parent compounds under various stress conditions, such as acidic, alkaline, oxidative, and photolytic environments.

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